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Effect of Four Dispersants on Biodegradation and Growth of Bacteria on Crude Oil

G. J. MULKINS-PHILLIPS AND JAMES E. STEWART

Environment Canada, Fisheries and Marine Service, Halifax Laboratory, Halifax, Nova Scotia B3J 2R3, Canada

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Four chemical dispersants, Corexit 8666, Gamlen Sea Clean, G. H. Woods Degreaser-Formula 11470, and Sugee 2 were examined singly and in individual combinations with Arabian Crude Oil (1:1 ratio) at 10 and 25 C for their effects on the growth of bacteria indigenous to local marine waters, the bacterial population composition, and biodegradation of crude oil; in addition, their emulsifying capacities, at approximately 24 C, were determined. None of the dispersants used alone were toxic even at relatively high concentrations (1.25%), although Gamlen Sea Clean and G. H. Woods Degreaser-Formula 11470 did cause an increase in the lag phase which was more pronounced at 10 than at 25 C; addition of the crude oil reduced the lag phase increase. All of the dispersants used alone supported good growth of microorganisms, but qualitative population shifts were caused by the dispersant-oil combinations. The degrees of degradation of the *n*-alkane fraction of the crude oil varied depending upon the dispersant used. Under these test conditions, only Sugee 2, which had the poorest emulsifying capacity, promoted *n*-alkane degradation compared with the values obtained by using the crude oil alone.

Chemical dispersants are often used to break up oil spills and disperse oil products which threaten to pollute shoreline areas. In the case of "Torrey Canyon," it was believed that such dispersants caused more harm to flora and fauna than did the polluting oil itself (13). Subsequently, a number of reports have appeared in the literature describing the toxic effects of chemical dispersants on various marine species (9, 12).

It has been assumed generally that the criteria for a good oil dispersant were adequate dispersing qualities and low toxicity to marine species (plankton, invertebrates, fish). Few reports exist which consider the effects of oil dispersants on microbial species, effects which might be important since it is assumed that biodegradation is the major natural process ultimately responsible for eliminating oceanic oil pollutants (1, 15). Gunkel (6) conducted experiments which suggested that the toxicity to bacteria of an oil dispersant was a function of the concentration, whereas Liu and Townsley (7) reported that polymerized lignosulfonates greatly increased the rate of kerosene degradation by soil bacteria. Similarly, Robichaux and Myrick (8) showed that crude oil degradation was enhanced by a number of dispersants. Atlas and Bartha (3), studying mineralization and biodegradation of Sweden crude oil, found that chemical dispersants increased the rate of mineralization but did not affect the extent of biodegradation. Thus, the results to date suggest that many but not all chemical dispersants increase the rate of degradation of oil.

Oil dispersants should be tested to ensure that they meet three further criteria: (i) they should be biodegradable; (ii) in the presence of oil, they must not be preferentially utilized as carbon source; and (iii) they must be nontoxic to indigenous bacteria. The experiments described in this report were carried out to provide information on some of these points for four chemical dispersants proposed for use in this geographical area.

The choice of the dispersants' concentration and the ratio of dispersant to oil was based on the following considerations. The recommended ratios for dispersant use generally fall in the region of 1 to 10 parts dispersant to 100 parts of oil. In practice the level most commonly used is the minimum which is seen to be effective. In Canada the use of approved dispersants is controlled (10), but here as elsewhere there is, as Canevari (5) states in discussing dispersant action, "a tendency to 'overtreat' and apply excessive amounts of chemical during an emergency." Thus, "treat ratios" can and have

exceeded 1:1 in certain applications. In addition, the dispersants, whether lipophilic or hydrophilic, are concentrated at the water-oil interface, thus aiding in dispersion to the degree that temperature, oil viscosity, and mixing energy permit. Thus, the dilution factor of importance will be the oil slick or droplet surface area, i.e., water-oil interface, and not the overall volume of oil. Consequently, the application of dispersants to water-borne oil will result in dispersant concentrations at the oil-water interface considerably higher than the "treat" ratios would suggest. Since the oil is dispersed rather than dissolved, these concentrations will remain to the extent that the oil droplet or slick remains intact. Biodegradation of either dispersant or oil is one obvious factor which can alter this relationship, but the microorganisms acting at the oil-water interface are subjected to higher concentrations of dispersants than are usually considered.

In practice, particularly for large masses of water-borne oil, mixing of the oil and dispersant is imperfect and fails to give a homogeneous mixture. Thus there would be a tendency for the dispersant to be distributed unevenly, i.e., the highest concentrations would be expected in the oil which is already in contact with water or is first brought into contact with water. If the temperature and viscosity are adverse (5) there is the distinct possibility of forming a water-in-oil emulsion (chocolate mousse), which along with the foregoing dilution considerations result in a further concentrating effect on the dispersant at the water-oil interface where the bacteria act.

Thus, we have chosen a dispersant concentration of 1.25% (vol/vol) and a ratio of dispersant to crude oil of 1:1 as being more realistic under field situations than those normally used and appropriate for gauging the effects of dispersants on bacterial action within the limits of the experimental design.

MATERIALS AND METHODS

Quantities of sea water (at 13 to 14 C) were collected aseptically from Halifax Harbor, an area tested previously and described (7a) as being chronically polluted with low levels of oil. Portions (40 ml) were dispensed into sterile flasks, and sterile solutions of NH_4NO_3 and K_2HPO_4/KH_2PO_4 were added aseptically to give concentrations of 875 mg of N per liter and 970 mg of P per liter and a final pH of 7.3. The chemical dispersants (1: Corexit 8666, Esso Chemicals Canada, Toronto, Ont.; 2: Gamlen Sea Clean, Gamlen Chemical Co., San Francisco, Calif.; 3: G. H. Woods Degreaser-Formula 11470, G. H. Woods Ltd., Toronto, Ont.; 4: Sugee 2, Handy Chemical Ltd., LaPrairie, Que.) were added to give concentrations each of 1.25% (vol/vol); Arabian crude oil was added where indicated at the same concentration, 1.25%. Growth control flasks were identical to the growth flasks but contained no added carbon (dispersants or oil); chemical control flasks were identical to the growth flasks and were treated identically in all respects except for the addition of NaN_3 (final concentration, 0.01 M).

Flasks were incubated on a refrigerated shaker bath (New Brunswick Scientific Co., New Brunswick, N.J.) at 120 rpm and either 10 or 25 C.

Total viable bacterial numbers were determined by the drop-plate method on Trypticase soy agar (BBL) plates containing 3% NaCl as described earlier (G. J. Mulkins-Phillips and J. E. Stewart, submitted for publication). Bacterial populations were analyzed and characterized to the genus level by methods outlined by Shewan (11).

The emulsifying capacity of the dispersants at room temperature (24 C) was estimated by measuring the turbidity of 1.25% oil plus 1.25% dispersant in nonenriched seawater. The combinations were mixed for 1.5 min (Vortex mixer); percent transmittance of the mixtures was measured at 545 nm on a Bausch and Lomb Spectronic 20 spectrophotometer during the 140-min period immediately following the mixing step. The procedure was similar to that described by Canevari (4) in which low percent transmittance values indicated good dispersing qualities.

In preparation for gas-liquid chromatographic measurements of the extent of biodegradation, growth flasks containing oil and oil plus dispersants 1 and 4 were extracted by a previously described modification (Mulkins-Phillips and Stewart, submitted for publication) of a method outlined by Soli and Bens (13). Oil plus dispersants 3 and 4 were chromatographed directly. Details of the equipment and operating conditions for gas-liquid chromatographic measurements were described previously (Mulkins-Phillips and Stewart, submitted for publication); the column was programmed between 100 and 300 C at 7.5 C/min. Normal alkane peak heights were measured on test and control chromatographs, and the percent degradation of each was determined; the results recorded were average values for the C_{17-28} range. This range was chosen because it was the area free of overlap with all the dispersants' alkane fractions and thus brought all values to a common base. The areas free from overlap with the individual dispersant-oil combinations were: C_{11}-C_{28} for oil plus dispersant 4, C_{16}-C_{28} for oil plus dispersant 1, C_{14}-C_{28} for oil plus dispersant 3, and C_{17}-C_{28} for oil plus dispersant 2. Individual calculations based upon these ranges were not materially different from those presented.

RESULTS

All four chemical dispersants, when added as sole carbon sources to enriched seawater, supported growth of the indigenous bacterial populations (Fig. 1, 3). The growth curves obtained for microorganisms which grew in the presence of dispersants 2 and 3 exhibited longer lag

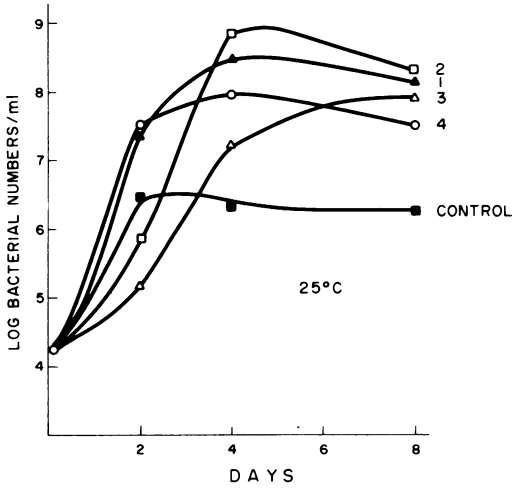

FIG. 1. *Growth of indigenous microorganisms on chemical dispersants (1.25%) at 25 C.*

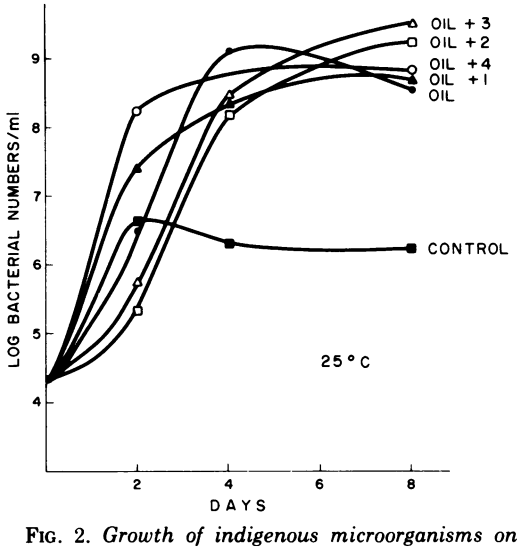

FIG. 2. *Growth of indigenous microorganisms on Arabian crude oil (1.25%) plus chemical dispersants (1.25%) at 25 C.*

phases which were more pronounced at 10 than at 25 C. This inhibition was overcome with time, and the stationary-phase population numbers for all four dispersants were similar. When dispersants 2 and 3 were incubated with Arabian crude oil, the lag phases noted above were reduced (Fig. 2, 4). Stationary-phase population numbers for all dispersants were not appreciably affected by the addition of oil.

Analyses of the bacterial populations which grew in the presence of oil and oil plus dispersants showed a predominance of *Pseudomonadaceae* and *Achromobacter* species (Table 1). The percentage of each species varied for each dispersant, indicating that the dispersants had an effect on the indigenous microbial population.

Turbidimetric measurements of the dispersants added to oil plus seawater illustrated the emulsifying capacity, which in decreasing order of effectiveness was 2 > 3 > 1 > 4 (Fig. 5). Dispersant 2 formed a very stable emulsion, whereas the effect of dispersant 4 was poor, giving an emulsion which was only slightly better than that obtained when the oil alone was mixed with seawater.

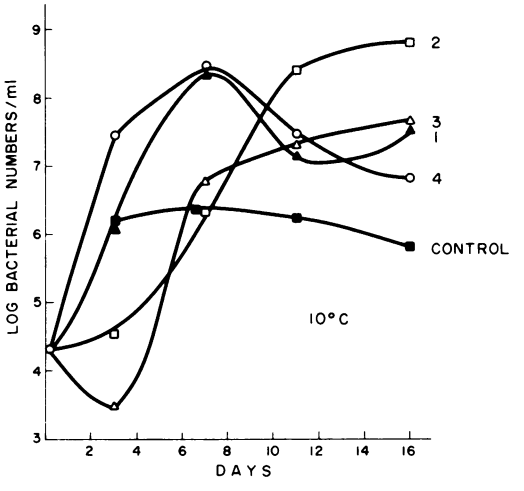

FIG. 3. *Growth of indigenous microorganisms on chemical dispersants (1.25%) at 10 C.*

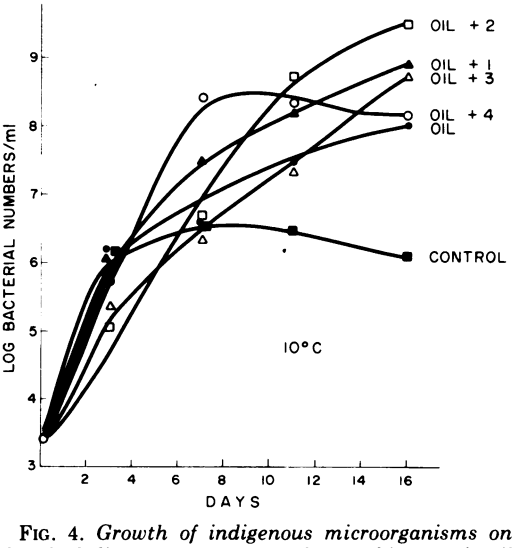

FIG. 4. *Growth of indigenous microorganisms on chemical dispersants (1.25%) plus Arabian crude oil (1.25%) at 10 C.*

TABLE 1. *Percentage of distribution of bacterial genera species growing on oil and oil plus dispersants at 25 C*

Day	Genera	Percentage of genera growing on					
		Control (no carbon added)	Oil	Oil + dispersant 1	Oil + dispersant 2	Oil + dispersant 3	Oil + dispersant 4
0	*Paracolon*	12.5	12.5	12.5	12.5	12.5	12.5
	Pseudomonas	21.0	21.0	21.0	21.0	21.0	21.0
	Vibrio	4.0	4.0	4.0	4.0	4.0	4.0
	Aeromonas	0	0	0	0	0	0
	Achromobacter	62.5	62.5	62.5	62.5	62.5	62.5
2	*Paracolon*	0	0	10.0	33.0	19.0	0
	Pseudomonas	62.0	70.0	75.0	8.0	56.0	47.0
	Vibrio	0	0	0	0	0	0
	Aeromonas	0	0	0	17.0	0	0
	Achromobacter	38.0	30.0	15.0	42.0	25.0	53.0
4	*Paracolon*	0	0	0	0	0	0
	Pseudomonas	54.0	10.0	36.0	100.0	100.0	40.0
	Vibrio	0	0	0	0	0	0
	Aeromonas	0	0	0	0	0	0
	Achromobacter	46.0	90.0	64.0	0	0	60.0
8	*Paracolon*	0	0	0	0	0	0
	Pseudomonas	30.0	61.0	40.0	100.0	100.0	52.0
	Vibrio	0	0	0	0	0	0
	Aeromonas	0	0	0	0	0	0
	Achromobacter	70.0	39.0	60.0	0	0	48.0

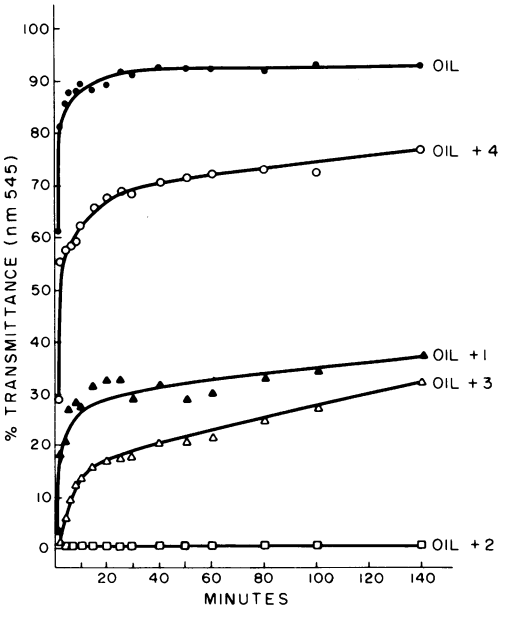

FIG. 5. *Effectiveness of chemical dispersants in forming emulsions with Arabian crude oil in seawater at room temperature (ca. 24 C).*

Measurements of the disappearance of the *n*-alkane fraction of the oil, which had been incubated with the different dispersants, demonstrated the extent to which degradation was affected by these dispersants. Incubation of oil with dispersants 1, 2, and 3 resulted in decreased degradation values (Table 2), whereas the presence of dispersant 4 resulted in a slight increase. In these particular experiments oil plus dispersant 3 gave low values for maximal population size, and extended lag phases were noted again for the incubation of oil with dispersants 2 and 3. Addition of dispersant 1, 2, and 4 to oil did not affect the ultimate population sizes. The degradation values obtained for oil and oil plus dispersants were, as expected, lower at 10 than at 25 C.

DISCUSSION

All four of the oil dispersants supported growth when used alone even at relatively high concentrations at both temperatures (Fig. 1, 3). None proved to be toxic, although dispersants 2 and 3 extended the lag phase of growth, an extension which was more pronounced at 10 C than at 25 C. In fact, at 10 C (Fig. 3) dispersant

TABLE 2. *Percent degradation of C_{17-28} n-alkane fraction of Arabian crude oil incubated with the chemical dispersants*

Sample	Incubation at							
	10 C[a]		10 C[a]		25 C[b]		25 C[c]	
	% Degradation	Maximal bacterial population	% Degradation	Maximal bacterial population	% Degradation	Maximal bacterial population	% Degradation	Maximal bacterial population
Oil	43	1.2×10^8 (day 16)	32	8.0×10^6 (day 16)	72	5.0×10^7 (day 6)	100	1.2×10^9 (day 4)
Oil Plus Dispersant 1	0	5.6×10^8 (day 16)	23	1.2×10^8 (day 11)	44	1.6×10^9 (day 6)	100	4.0×10^8 (day 4)
Oil Plus Dispersant 2			12.5	3.4×10^8 (day 11)			94	2.8×10^9 (day 8)
Oil Plus Dispersant 3			29	2.8×10^7 (day 16)			7	2.2×10^6 (day 8)
Oil Plus Dispersant 4	49	4.0×10^8 (day 7)	36	1.8×10^8 (day 11)	83	4.0×10^8 (day 6)	100	3.8×10^8 (day 4)

[a] Incubation for 16 days, after which the oil was analyzed by gas-liquid chromatography (GLC). Trials were in duplicate.

[b] Incubation for 6 days, after which the oil was analyzed by GLC. Trials were in duplicate.

[c] Incubation for 8 days, after which the oil was analyzed by GLC. Trials were in duplicate.

3 had an inhibitory effect in the early stages of growth period, possibly resulting from a volatile fraction which had to evaporate before good growth could occur, similar to the findings of Atlas and Bartha (2) with Sweden crude oil at lower temperatures. These extensions of the lag phase or inhibitory effects were markedly reduced when Arabian crude oil was added along with the dispersant (Fig. 2, 4), possibly through dilution or competitive action.

Population shifts in the presence of dispersants were observed (Table 1). Further work would be required to determine the significance of these shifts, but it is interesting to note that dispersants 2 and 3 shifted the population to one based entirely on pseudomonads; these two dispersants produced an extended lag phase (Fig. 1, 3) and both retarded biodegradation at 10 and 25 C, although to entirely different degrees. Dispersant 1 retarded biodegradation much more at 10 C than at 25 C. The retardation of biodegradation illustrated in Table 2 might result not from inhibition but rather from a preferential use of the dispersants by the microorganisms, except in the case of dispersant 3 at 25 C where it would appear that growth reduction was a more likely cause.

In this regard it is interesting to compare the results Atlas and Bartha (3) obtained with dispersant 1 (Corexit 8666) and others. They used a 1% (vol/vol) concentration of oil and a dispersant-oil ratio of 1:20 and observed an approximately 17% increase in carbon dioxide evolution with Corexit 8666, Shell Oil Herder #3, and Smith Oil Herder over that obtained with oil alone during the course of the 28-day trial. This was interpreted as increased mineralization of the oil in the presence of the dispersants. When biodegradation was determined chemically at the end of the trial, however, the final percent biodegradation values for oil alone was virtually identical with those for oil plus dispersants, a finding which, omitting dispersant 3 values, was similar to our own results (Table 2). It is probable that the increase in carbon dioxide evolution observed by Atlas and Bartha (3) actually resulted from mineralization of the dispersant rather than the oil. Thus, two separate experiments with two different Corexit 8666 concentrations, dispersant-oil ratios, and bacterial populations appear to have given surprisingly similar results.

Dispersant 4, which was the poorest emulsifier (Fig. 5), was the only dispersant which actually stimulated biodegradation. Obviously, if dispersion alone was the controlling factor in microbial degradation of spilled oils, the effectiveness of the dispersants would be rated, in

decreasing order of effectiveness, as 2 > 3 > 1 > 4. This order would be that expected to promote a cosmetic dispersion and apparent disappearance of the oil. Judging by the results of our experiments, the order in which the dispersants would enhance the biodegradation of the *n*-alkane fraction would be 4 > oil alone > the other three dispersants.

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